Note added March 9.

Since the above was communicated to the Society we have made, at the suggestion of Mr. Langley, some further experiments upon the action of nicotine, employing cats instead of dogs (since in his experience the action of nicotine upon dogs is much more uncertain and difficult of interpretation). We find that in the cat a small dose of nicotine (1 to 3 milligrams) temporarily abolishes the effect of stimulating the nerve-roots, whilst only slightly diminishing the effect of stimulating the splanchnics; this being the same result as that above described upon the dog. On the other hand, a large dose of nicotine (10 to 12 milligrams) entirely abolishes the effects of stimulating both the nerve-roots and the splanchnics, whilst the nerves which pass to the spleen along with its blood-vessels are still freely excitable. It is clear, therefore, from the last result that large doses of nicotine cause a complete block between the splanchnics and the splenic nerves, *i.e.*, in the semilunar ganglion; so that there is, in all probability, a cell station in this ganglion for all the splenic fibres. On the other hand, as we have seen above, a block is apparently produced by small doses of nicotine between the nerve-roots and the splanchnics, *i.e.*, in the ganglia of the chain. Whether this is really due, as we have suggested in the text, to the existence of another cell-connection in these ganglia, or whether, as Mr. Langley has suggested to us, it is capable of another interpretation is a question which we propose to reserve for a detailed communication of our experiments in the 'Journal of Physiology,' and we will also defer until that communication reference to a paper by Bulgak on the innervation of the spleen, which appeared in Virchow's 'Archiv,' vol. 69, 1877, and which had escaped our attention.

II. "A Method for rapidly producing Diphtheria Antitoxines. Preliminary Note."* By G. E. Cartwright Wood, M.D., B.Sc. Communicated by Dr. Pye-Smith, F.R.S. Received February 20, 1896.

The method for producing antitoxines, described in this preliminary communication, is the outcome of an investigation into the action of the products of the diphtheria bacillus on which I have been engaged

* The investigation has been carried out in the laboratories of the Royal College of Physicians and Surgeons, and I should like here to express my great indebtedness to the Laboratories Committee for the facilities there afforded to me. I must also thank them and, through them, the Honourable Goldsmiths' Company, from whose Research Fund a grant was placed at my disposal.

for more than a year. The toxines described by Continental observers have been those obtained from broth cultivations of the organism, where we cannot expect the products, even if identical in nature, to be present in the same proportions as when vegetating in the living body of an animal. More than eight years ago Hueppe* and I pointed out that we must grow the pathogenic organism on a natural albumen if we wished to obtain the toxines proper of the microbe; and working on this principle by inoculating hens' eggs with Koch's cholera bacillus we succeeded in producing much more powerful poisons than had been previously obtained. The toxines produced by the action of the diphtheria bacillus on albumen have been minutely investigated by Sidney Martin, and the occurrence of the same substances in the bodies of patients who have succumbed to the disease has proved the correctness of this view. In this communication I shall not enter into the precise nature of the products in question, as my present purpose is to describe a practical method of rapidly producing powerful diphtheria antitoxines.

In these experiments two species of diphtheria toxine were made use of: firstly, the ordinary toxine produced by the organism in peptone broth; secondly, the substances present in serum-broth cultivations which had been filtered and heated up to 65° C. [In the former the active principle consists almost entirely of the so-called ferment toxine, while in the latter this has been destroyed by heating up to 65° C., so that its action must depend on the presence of other substances.]† The medium employed for the production of the serum toxine was ordinary peptone broth, to which an addition of 10 or 20 per cent. blood serum or plasma had been added; where the latter was used the broth was previously decalcified to prevent coagulation. As a rule the broth was inoculated with a virulent diphtheria culture some three or four days previous to the addition of the serum or plasma, and then incubated at a temperature of 37° C. for at least three or four weeks. Before being used for injection it was subjected to a temperature of 65° C. for about an hour and then filtered through a sterilised Chamberland candle to remove the bodies of the bacilli. This fluid will be spoken of subsequently as "serum" toxine in contradistinction to the ordinary poison, which will be spoken of as "broth" toxine. The serum toxine is characterised chiefly by giving rise to little local irritation but marked febrile reaction which is still more pronounced when the injection is repeated. As the diphtheria albumose described by Sidney Martin was characterised by precisely these properties, in all probability the potency of the serum toxine depends on its presence. In addition to these properties, however, it was found that animals which had been subjected to its

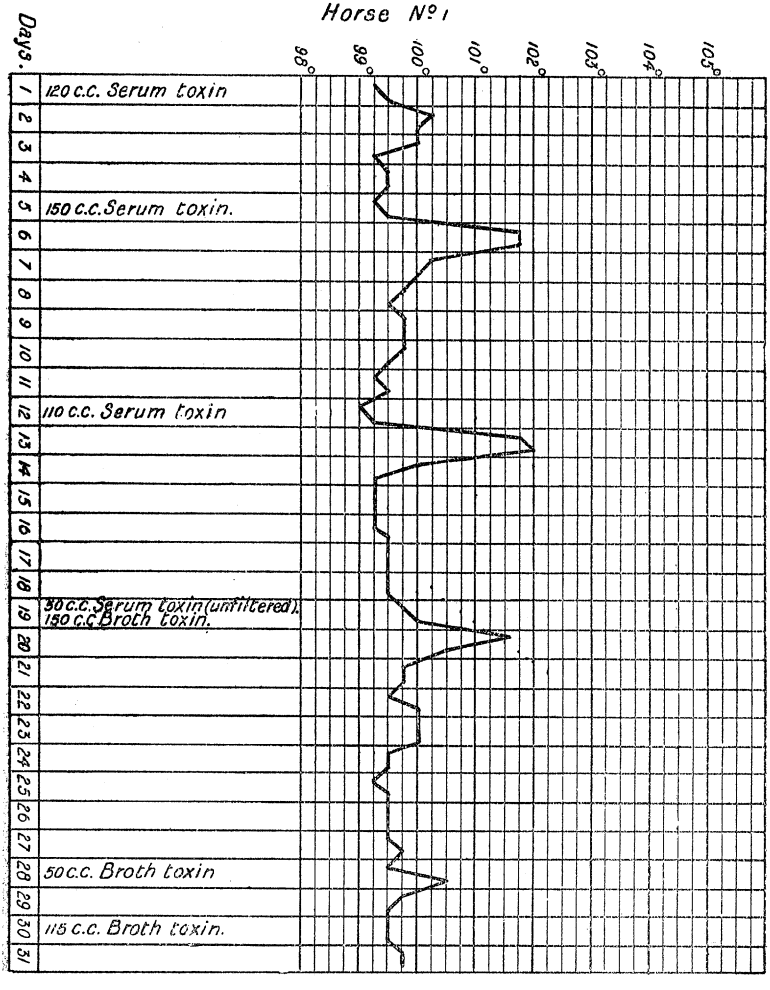
* 'Cent. f. Bakt.,' No. 5, 1884.

† The passages in brackets were added after the reading of the paper.

action were rendered more or less refractory to subsequent infection, and this suggested the possibility of its application as a means of shortening the preliminary treatment which a horse must undergo before it can receive the large doses of broth toxine which are usually necessary for the production of antitoxine of any strength.

The first horse (No. 1) was treated on the same general principles which are adopted in immunising guinea pigs ; that is to say it receives a certain quantity of the vaccinating substance and after the lapse of ten or fourteen days it is subjected to the action of the microbe or

Table I.



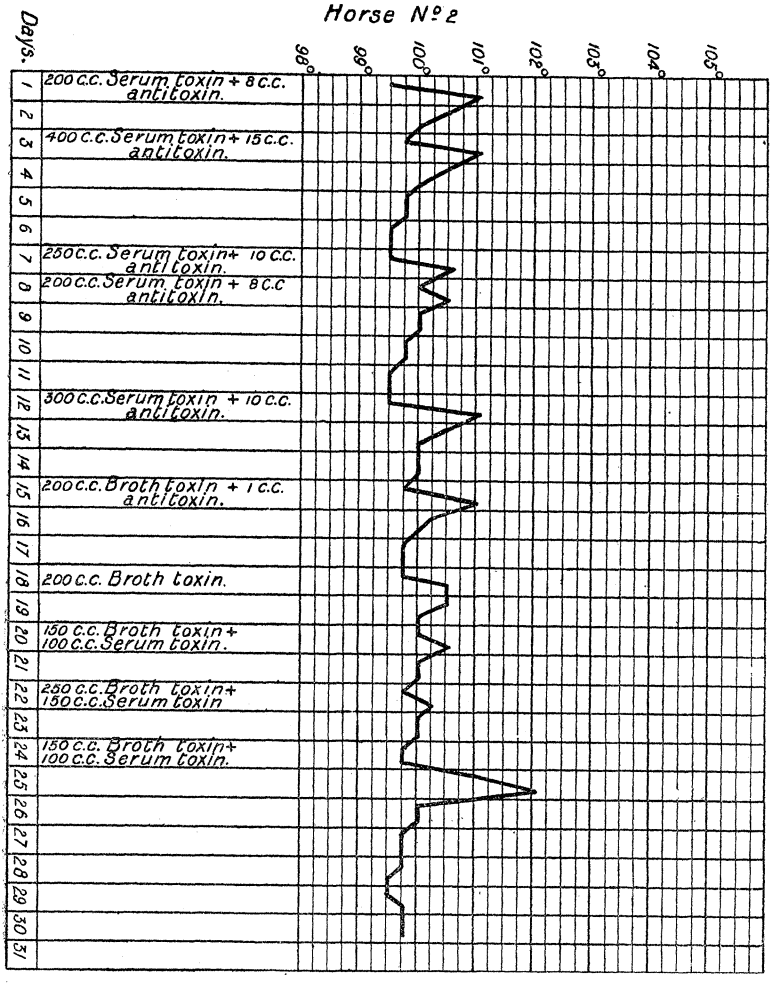
toxine. During the first twelve days this horse received 380 c.c. of serum toxine (see Table I) spread over three injections on different dates. On the nineteenth day of the experiment it received 50 c.c. of unfiltered serum toxine (sterilised at 65° C.) and 150 c.c. of broth toxine, of which one half c.c. killed a 500 gramme guinea-pig in forty-eight hours. The unfiltered serum toxine unfortunately gave rise to a small abscess which greatly impeded the treatment, and subsequent experience has convinced me that the bodies of the bacilli which gave rise to this irritation must be removed by filtration if the process is to be carried out smoothly and successfully. On the 28th day it received 50 c.c. of the same broth toxine, and on the 30th day another injection of 115 c.c.

The horse was then bled on the thirty-second day of treatment, and the serum was found to possess the strength of ten normal units, that is to say, 1/100th of a c.c. protected a 250 gramme guinea-pig against ten lethal doses of broth toxine. As this strength is only attained by Roux's method after at least ten weeks' treatment, it was evident that the serum toxine had considerably shortened the process. It may be mentioned that the horse, which had been in very poor condition at the beginning of the treatment, steadily improved during the month. The experiment was not carried further with this horse, which was then subjected to the ordinary method for producing antitoxines, when it reacted in every respect like an animal which had been under the usual treatment for several months.

In the case of the next horse (No. 2) it seemed safe to inject much larger quantities of the serum toxine, but to avoid the risk of constitutionally injuring the animal the addition of a certain amount of antitoxine was made. During the first twelve days (Table II) 1350 c.c. of serum toxine had been injected and mixed with this 50 c.c. of antitoxine obtained from the previous horse, each c.c. of which contained ten normal units. During the next week 550 c.c. of broth toxine (of which 1/4 c.c. killed a 500 gramme guinea-pig in forty-eight hours) was injected, spread over three injections, and to the last of these 100 c.c. of serum toxine was added. In the succeeding week it received 400 c.c. of the same broth toxine mixed with 250 c.c. of serum toxine in two injections. On the thirtieth day the animal was bled (3/4 of a litre) and the antitoxic value of its serum estimated. It was found that 1/100, 1/200, 1/300, 1/400, 1/500, and finally 1/1000th of a c.c. protected completely against ten lethal doses of the toxine. After six weeks of treatment 1/1600th of a c.c. protected against ten lethal doses, while on the ninth week 1/2500th of a c.c. sufficed.

In this experiment, which furnished such brilliant results, as has been stated antitoxine was mixed with the serum toxine injected, but that this was not at all necessary was shown by a subsequent experi-

Table II.



ment in which another horse (No. 3) received no less than 2180 c.c. serum toxine in the space of a fortnight without any apparent injury. The serum of this animal was tested during the first and second weeks, before it had received any broth toxine, and it was found that 1/300th and 1/500th of a c.c. respectively protected guinea-pigs against ten lethal doses. On the third week 1/850th of a c.c. protected, and at the end of a month 1/1250th of a c.c. sufficed. [The large amount of antitoxine produced during the first two weeks, when the horse received serum toxine alone, was quite unexpected, and leaves still

unsettled the question as to how far the acquired immunity produced by the serum toxine is due to increased tissue resistance, or to the presence of the antitoxine in the fluids of the body.] These results are shown in the appended table (Table III).

Table III.—Horse No. 3.

	Antitoxic value of serum.	Amount of toxines injected.
7th day.....	$\frac{1}{300}$ c.c.	1200 c.c. serum toxine.
14th day.....	$\frac{1}{500}$ c.c.	980 c.c. serum toxine.
21st day	$\frac{1}{850}$ c.c.	650 c.c. serum toxine and 1050 c.c. weak broth toxine.
28th day.....	$\frac{1}{1250}$ c.c.	1100 c.c. serum toxine and 1200 c.c. stronger broth toxine.

Although the serum toxine had been used primarily in the expectation of rapidly immunising the animal, and thus shortening the necessary period of treatment, the very high antitoxic value of the serum obtained from horses Nos. 2 and 3 suggested that the method might be applied effectively at a later stage. For the purpose of testing this a number of horses which had been under the ordinary treatment for from six to nine months were very kindly placed at my disposal by Dr. Woodhead. In the first experiments 200 or 300 c.c. of the serum toxine was mixed with the ordinary broth toxine, and injected as usual. The results obtained, although slightly better, were not at all so marked as one might have expected. On examining more in detail the horses in which the best results had been obtained, it was found that these had been under more or less continuous treatment with the serum toxine, both toxines being injected as frequently and in as large amounts as possible. Guided by these facts four of the horses (Nos. 4, 5, 6, 7) received one evening each 300 c.c. of serum toxine, and on the following morning an injection of weak broth toxine. Although the quantity of weak broth toxine would, under normal conditions, have produced hardly any effect, it produced on this occasion most marked local and constitutional reactions. During the remainder of the week these horses received injections of weak broth toxine each day, or on alternate days according to their condition, and on each occasion these gave rise to quite definite constitutional and local reactions. During the following week an injection of 300 c.c. of serum toxine was introduced, succeeded by similar quantities of weak broth toxines, as in the previous week. [It will be observed that all these horses received practically the same quantities of toxine, with the exception of horse No. 5, in which the

injections were stopped earlier than the others, owing to its temperature showing a tendency to remain permanently elevated. In horses Nos. 6 and 7 the production of antitoxine was much less than in Nos. 4 and 5, and this is, no doubt, to be ascribed to the injections producing much less marked reactions, owing, apparently, to their greater refractoriness. In all probability, however, this condition might have been overcome by the use of larger injections and stronger broth toxine.] The rise in antitoxic value of the serum of these horses is shown in the appended table (Table IV).

Table IV.

	Strength of serum before treatment.	Strength of serum after 16 days treatment.	Amounts of toxines injected during the 16 days.
Horse No. 4	$\frac{1}{600}$ c.c.	$\frac{1}{2000}$ c.c.	650 c.c. serum toxine and 2350 c.c. weak broth toxine.
Horse No. 5	$\frac{1}{400}$ c.c.	$\frac{1}{1500}$ c.c.	600 c.c. serum toxine and 1800 c.c. weak broth toxine.
Horse No. 6	$\frac{1}{300}$ c.c.	$\frac{1}{750}$ c.c.	650 c.c. serum toxine and 2350 c.c. weak broth toxine.
Horse No. 7	$\frac{1}{400}$ c.c.	$\frac{1}{750}$ c.c.	650 c.c. serum toxine and 2350 c.c. weak broth toxine.

These results indicate very strikingly that the rapid production of antitoxine depended, at any rate in great part, on the cumulative action of the toxines by means of which the animal was kept in a chronic condition of local and constitutional reaction. It is probable that we produce in this way the earlier stages of that condition of "supersensitiveness" described by Behring, in which an animal whose blood may be charged with the most powerful antitoxines, suffers the most profound constitutional disturbance on the introduction of even the smallest quantities of toxine, while at the same time the temperature of the animal may remain for months permanently elevated above the normal. By taking advantage, however, of this cumulative action in its earlier effects, we are furnished with a means of easily producing much more powerful antitoxines than is otherwise possible, and it is probable that by the use of stronger broth toxine the method may be carried still further in this direction. This cumulative action may also be taken advantage of to obviate that most troublesome occurrence where an animal becomes apparently absolutely refractory, ceasing to react to the toxine and failing to produce antitoxine, so that it has to pass out of use for the purpose of producing the curative serum. Some preliminary experiments have, however, indicated that

this cumulative action may be produced still more markedly by the use of other toxines than those elaborated by the diphtheria bacillus, a result which I was quite prepared for, as Woodhead* and myself had in a previous communication drawn special attention to this summative action of bacterial products.

Although this part of the investigation is still quite incomplete, an application of this principle, which may be of importance, may be here suggested. The excessively costly nature of snake venom and the practical difficulties of obtaining it in sufficient quantities have been a great obstacle to the immunisation of the larger animals or the production of anti-venines of any high degree of strength. It is probable that by the use of other toxines in the later stage the quantities of the costly snake venom necessary may be greatly lessened.

[In this preliminary communication I have dealt with horses which have been under treatment only for a short period, and have shown that antitoxines at least as strong as the best in use can be quickly and easily produced, but I have every reason to believe that, under more prolonged treatment, much more powerful diphtheria antitoxines can be obtained than has been previously possible.]

In conclusion it may be said that the following advantages may be claimed for the use of the toxines in the way I have described.

1. That powerful diphtheria antitoxines can be produced without risk in a much shorter period of time than has been previously possible.

2. That much more powerful antitoxines can be easily produced so that the amount necessary to be injected into a patient can be greatly reduced, and one of the great objections to its introduction into private practice in this country may be removed.

3. That the greater strength of the serum will permit of the patient receiving at the beginning of treatment a sufficient quantity of the serum at one injection, when, as is universally recognised both by animal experiment and clinical experience, its curative action is exerted most markedly.

I must acknowledge my extreme indebtedness to the Director of the Laboratories, Dr. Sims Woodhead, for much invaluable advice and assistance during the course of this investigation.

* "On the Antidotal and Summative Actions that the Products of Bacteria exert on the Course of Infective Disease," 'Lancet,' February 22, 1890.